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Localization and function of five glutamate transporters cloned from the salamander retina

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Abstract

Glutamate is the major excitatory neurotransmitter in the vertebrate retina. Native glutamate transporters have been well characterized in several retinal neurons, particularly from the salamander retina. We have cloned five distinct glutamate transporters from the salamander retina and examined their localization and functional properties: sEAAT1, sEAAT2A, sEAAT2B, sEAAT5A and sEAAT5B. sEAAT1 is a homologue of the glutamate transporter EAAT1 (GLAST), sEAAT2A and sEAAT2B are homologues of EAAT2 (GLT-1) and sEAAT5A and sEAAT5B are homologues of the recently cloned human retinal glutamate transporter EAAT5. Localization was determined by immunocytochemical techniques using antibodies directed at portions of the highly divergent carboxy terminal. Glutamate transporters were found in glial, photoreceptor, bipolar, amacrine and ganglion cells. The pharmacology and ionic dependence were determined by two-electrode voltage clamp recordings from *Xenopus laevis* oocytes which had previously been injected with one of the glutamate transporter mRNAs. Each of the transporters behaved in a manner consistent with a glutamate transporter and there were some distinguishing characteristics which make it possible to link the function in native cells with the behavior of the cloned transporters in this study.

Keywords: Glutamate transporters; Cloned transporters; Salamander retina

1. Introduction

Glutamate is the predominant excitatory neurotransmitter throughout the vertebrate central nervous system, including the retina. Photoreceptors, bipolar cells and ganglion cells are all thought to release glutamate in a vesicular manner [1,2]. The termination of glutamatergic transmission requires the removal of glutamate from the extracellular space by means of excitatory amino acid transporter (EAAT) molecules, present in both glial cells and neurons. These glutamate transporters are capable of concentrating glutamate levels in the cell by several orders of magnitude by coupling the transport of glutamate with the electrochemical energy produced by the cotransport of 2–3 sodium ions and one proton, and the counter-transport of one potassium ion [3].

Glutamate transporters have been reported to be present in retinal glial cells [4,5], cone and rod photoreceptors [6,7] and bipolar cells [8]. Because the transport of glutamate is electrogenic (i.e. the uptake of glutamate is associated with a net inward movement of charge), these transporters have been well characterized using electrophysiological techniques [9]. They all share several similarities: transport of glutamate requires the presence of extracellular sodium; transport is voltage-dependent, with the greatest uptake at the most negative potentials; glutamate is transported stereospecifically (only the L-form is transported) whereas both L- and D-aspartate are transported equally well; and transport is partially inhibited by the transporter blocker dihydrokainate and by the substrate inhibitors *threo* β-hydroxyaspartate (THA) and L-*trans*-pyrollidine-2,4 dicarboxylic acid (tPDC).

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Table 1
Summary of glutamate transporter subtypes currently characterized

Subtype	Human names	CNS localization[a]	Cell types[a]	Chloride conductance	Rat retina localization[b]
GLAST	EAAT1	Cerebellum, some throughout CNS	Glial	Some[c]	Mueller cells
GLT-1	EAAT2	Throughout CNS	Glial	Some[c]	Some cones, some bipolar and amacrine cells
EAAC1	EAAT3	Throughout CNS	Neuronal	Some[c]	Horizontal, amacrine, ganglion and some bipolar cells
EAAT4	EAAT4	Cerebellum	Neuronal	>90%[d]	?
EAAT5	EAAT5	Retina	Both	>90%[e]	?

[a] [30,35].
[b] [24].
[c] [23].
[d] [14].
[e] [15].

Several distinct isoforms of glutamate transporters have been cloned [10–15] and in human these isoforms are termed EAAT1, EAAT2, EAAT3, EAAT4 and EAAT5. The glutamate transporters are membrane glycoproteins whose topology has not been fully determined, but current estimates suggest that there are ten transmembrane domains [16,17]. A recent study concluded that GLAST, GLT-1 and EAAC1 (homologues of EAAT1, EAAT2 and EAAT3, respectively) form homomultimers [18] but it is not yet known whether heteromultimers exist.

One interesting feature of retinal glutamate transporters is the presence of a chloride conductance associated with the transport of glutamate. For photoreceptors and bipolar cells, this conductance accounts for the majority of the glutamate-elicited current [7,8,19,20] but a chloride conductance is also present in retinal glial cells [21,22]. The chloride conductance shares all of the same pharmacological and ionic dependence properties described above with the high-affinity glutamate transporter, and it has been hypothesized that this conductance is in some way integral to the native glutamate transporter molecule [6–8,20–22]. The idea of a transporter with channel-like properties runs contrary to the accepted dogma for how a transporter works but recent studies indicate that this is a common feature seen in all five known glutamate transporter clones. As indicated in Table 1, some chloride conductance is associated with the clones EAAT1–EAAT3 [23] and the glutamate-evoked currents in EAAT4 and EAAT5, as in photoreceptors and bipolar cells, is predominantly a chloride current [14,15].

Work performed recently by Rauen et al. [24] on rat retina focused on the presence and localization of the clones GLAST, GLT-1 and EAAC1 (rat and rabbit homologues for human EAAT1, EAAT2 and EAAT3, respectively). All three of these transporters have been found throughout the rat retina, in retinal glial cells as well as in retinal neurons. The results of the localization studies, as well as their chloride conductance properties, is summarized in Table 1. One significant finding is that although there is physiological evidence for a large chloride conductance associated with glutamate transporters in photoreceptors, none of the three transporter clones studied mimics this behavior.

An obvious conclusion from this study is that there must be more than three subtypes of glutamate transporter present in the vertebrate retina. We therefore decided to probe a salamander retina cDNA library that we constructed to search for additional glutamate transporter subtypes. We found five distinct clones in the salamander retina, which we have termed sEAAT1, sEAAT2A, sEAAT2B, sEAAT5A and sEAAT5B. In this paper, we will examine the localization of these five transporters using immunocytochemical techniques, and the pharmacology and ionic dependence of these clones expressed in *Xenopus* oocytes using electrophysiological techniques. As will be shown, at least one of the five transporters behaves like the transporter in photoreceptors, and at least two of the five transporters are localized in photoreceptors.

2. Methods

2.1. Molecular cloning and sequence analysis

Complimentary DNAs (cDNAs) encoding five different glutamate transporter gene products were isolated from the salamander retina cDNA library. A complete description of the library construction and screening protocol has been published elsewhere [25]. Briefly, the retinal λZAP II library was screened with human EAAT cDNAs under reduced stringency conditions, and with specific salamander PCR-derived cDNA products under high stringency conditions, to isolate clones for full length coding sequences. Double-stranded DNA from the recovered pBluesript plasmids (Stratagene)

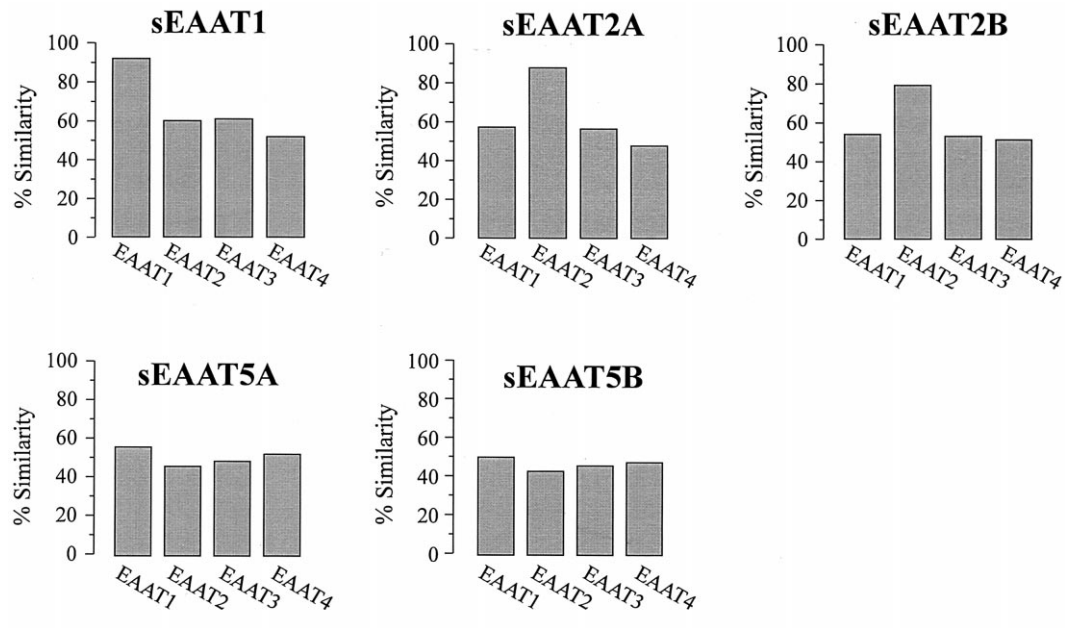

Fig. 1. Sequence similarity plots. Amino acid sequences predicted for each salamander glutamate transporter has been compared to the amino acid sequences of the human EAAT1–EAAT4. Percentage sequence similarity, a measurement of amino acid identity or conservative substitution at each position, as determined over the complete coding region using an optimal alignment. sEAAT5A and sEAAT5B stand out as a class distinct from EAAT1–4.

was sequenced using oligonucleotide primers, PRISM PCR-based sequencing reactions (Applied Biosystems) and an Applied Biosystems 373 Stretch DNA Sequencer. Nucleotide sequence data analyses and amino acid sequence similarity comparisons were performed using MacVector (International Biotechnologies).

2.2. Tissue and cell preparation

Eyes were dissected from salamanders and the cornea and lens were removed. The resulting eyecups were placed in a 4% paraformaldehyde PBS solution (pH 7.4) overnight at 4°C. Eyecups were then transferred to a 30% sucrose solution for another 8–12 h for cryoprotection and embedded in OCT with 3% glycerol. Frozen eyecups were sectioned onto Cell-Tak (Collaborative Biomedical Products) coated slides at 12 μm thickness using a cryostat. Sections were post-fixed in paraformaldehyde and permeabilized using 0.1% triton for 1 h prior to incubation with the antibody.

2.3. Immunocytochemistry

Antibodies were made to highly diverse regions of the intracellular carboxy terminal using GST fusion proteins for sEAAT1, sEAAT2A, sEAAT2B and sEAAT5A, and the crude serum was purified using affinity columns coupled to the fusion protein. A peptide antibody was made for sEAAT5B and was purified commercially. The antibodies do not cross-react, as determined from immunoblot experiments on oocytes expressing one of the five different clones (data not shown). The sections were incubated in the primary antibodies overnight at 4°C. After washing in PBS, the sections were incubated in a Cy5 conjugated donkey anti-rabbit IgG for at least 2 h at room temperature. As a control, fusion protein or peptide was present with the primary antibodies at 2–10 times the concentration of antibody. Thus the primary antibody binds entirely to the added protein, and the Cy5 tagged-secondary antibody does not bind to the tissue (Fig. 2, right column). Images shown are 0.5 μm thick optical slices obtained using a BioRad confocal microscope.

2.4. Solutions

Normal extracellular solution consisted of (in mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$ and 5 $Na_{\frac{1}{2}}$HEPES, pH 7.5. For sodium-free solutions, 96 mM N-methyl D-glucamine chloride (NMDGCl) replaced 96 mM NaCl and the pH was adjusted using NMDG. For chloride-free extracellular solutions gluconate salts replaced all chloride salts. Nitrate extracellular solution contained 96 mM $NaNO_3$ replacing 96 mM NaCl. Amino acids and transporter blockers were added directly to the appropriate extracellular solution.

2.5. Xenopus expression and electrophysiological recordings

Diluted mRNA (50 nl) transcribed from the linearized DNA was microinjected into defolliculated

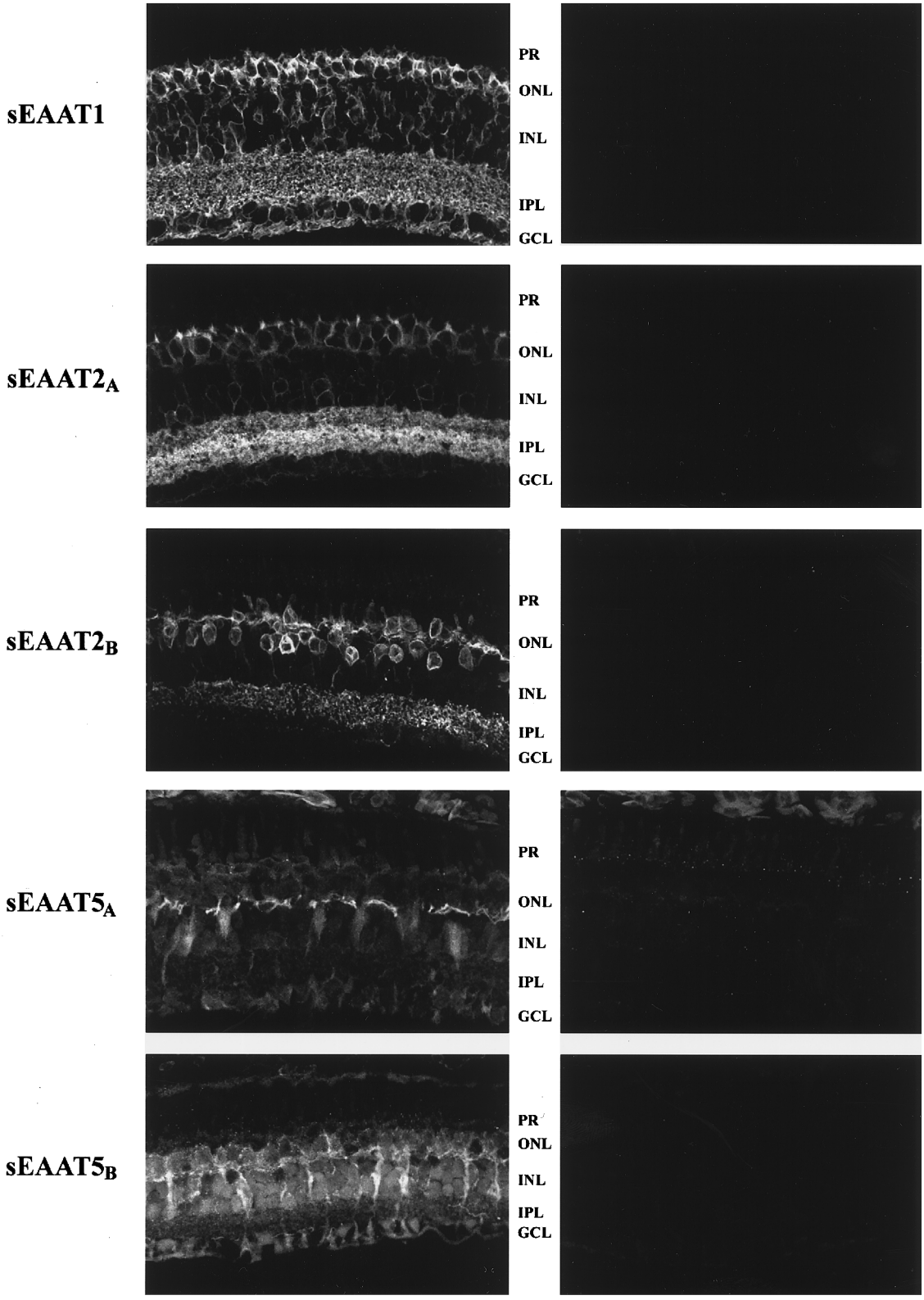

Fig. 2. Immunofluorescence confocal images showing localization of the five salamander glutamate transporter clones (left). Control figures, performed by incubating the sections in primary antibody simultaneously with fusion protein (or peptide for sEAAT5B) are shown in right column. PR, photoreceptors; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL ganglion cell layer.

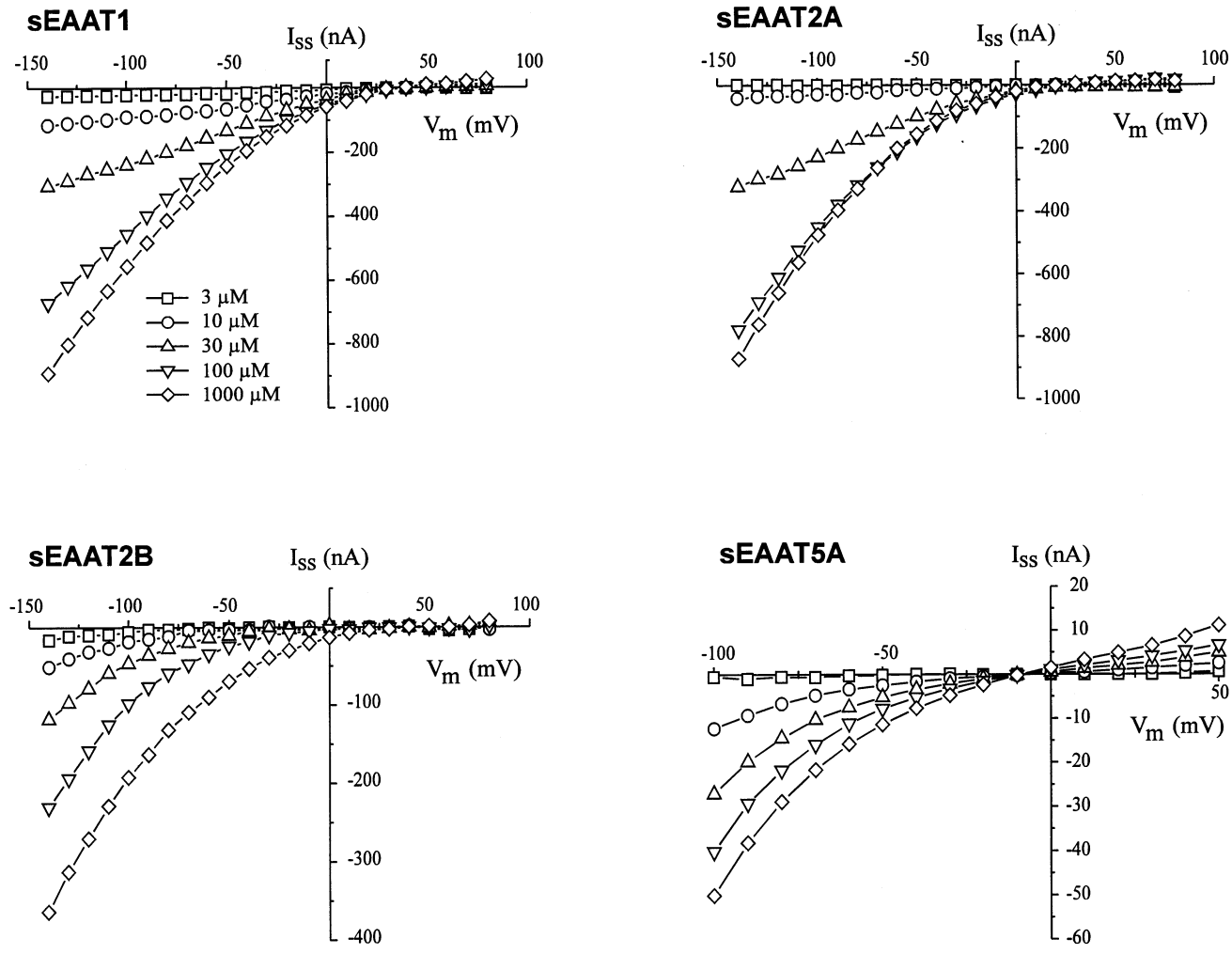

Fig. 3. Concentration and voltage-dependence of glutamate-elicited currents in four EAAT subtypes. Plotted are steady-state currents (I_{ss}) elicited in response to various concentrations of L-glutamate from 3 to 1000 μM at various membrane potentials (V_m). Concentrations are indicated in the legend. Each set of curves represents a typical cell. Similar results were obtained in four other cells for each subtype.

stages V–VI oocytes dissected from *Xenopus laevis* 3–7 days prior to recording. Two-electrode voltage clamp recordings were made using electrodes filled with 3 M KCl having resistances of less than 1 KOhm. The signals were amplified using a GeneClamp 500, digitized using a Digidata 1200, and controlled via a computer running the pClamp6 suite of programs (Axon Instruments). The membrane potential was held at −30 mV and stepped through a range of command potentials from −140 to +80 mV for 100 ms. Current-voltage (I–V) curves were constructed by obtaining the steady state current during the last 20 ms of the command step in the presence and absence of added drugs, and the difference I–V data is shown. Dose-response curves were fit to the Michaelis Menten equation using the Levenberg-Marquardt algorithm provided in Microcal's Origin.

3. Results

3.1. Sequencing and cloning

Five glutamate transporters were cloned from the salamander retina. To determine in which subfamily of glutamate transporter each of the five clones belongs, the amino acid sequence similarity of these clones were compared with the human clones EAAT1-EAAT4 (Fig. 1). sEAAT1 clearly belongs in the EAAT1 subfamily, as the conserved similarity between the two sequences is 80%. In fact, the epitope region in the carboxy terminal of EAAT1 is sufficiently close to the corresponding region on sEAAT1 that we were able to use the EAAT1 antibody on the salamander retina. The clones sEAAT2A and sEAAT2B most closely resemble EAAT2, having a similarity of 80 and 62%, respectively. Although both are most similar to EAAT2,

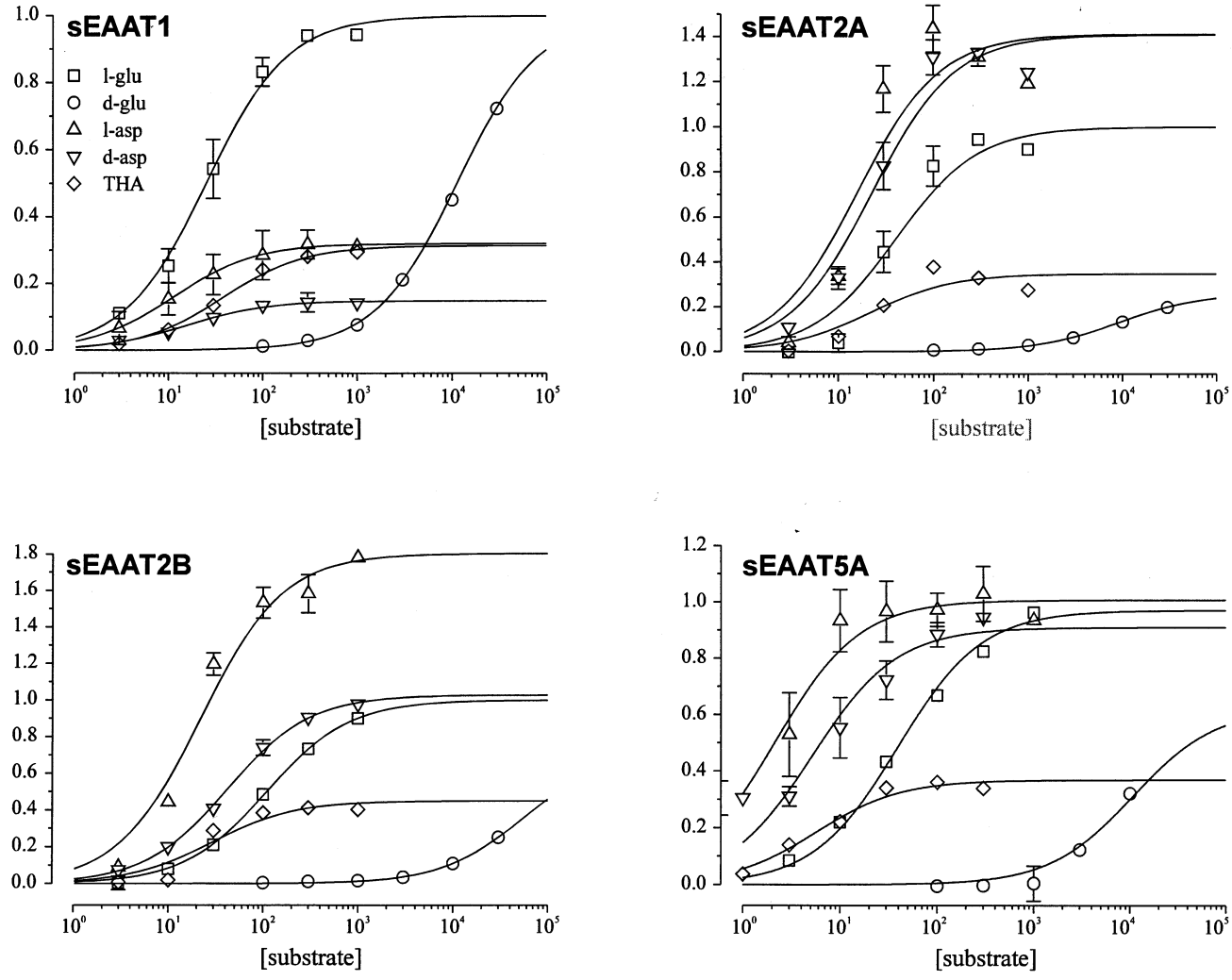

Fig. 4. Dose response curves from four EAAT subtypes for various substrates, normalized to the response to L-glutamate. Curves represent best fit to the Michaelis Menten equation. Fitting parameters (I_{max} and EC_{50}) are shown in Table 2. Error bars represent standard error of the mean.

sEAAT2A and sEAAT2B are distinct from each other; they share a sequence similarity of 61%, and as will be demonstrated they have a unique localization and functional properties. The clones sEAAT5A and sEAAT5B are most similar to the EAAT1 and EAAT4 subfamilies. However, the amino acid sequence similarity is never greater than 40% for either, and is therefore different enough that we are compelled to create a new subfamily, the EAAT5 family, of which both are members. sEAAT5A and sEAAT5B are only 58% similar to each other, and as will be shown below are sufficiently different in function as to form two distinct members the EAAT5 subfamily. The human homologue of sEAAT5, EAAT5, has recently been cloned and characterized [15]. sEAAT5A has 85% amino acid sequence similarity with EAAT5, while sEAAT5B has 65% sequence similarity (not shown).

The salamander cDNA library was also probed with primers designed to specifically pick up the EAAT3 and EAAT4 subtypes, but no positive result was obtained. This is consistent with negative results obtained from immunoblots using EAAT3 and EAAT4 fusion protein antibodies (unpublished observations).

3.2. Immunocytochemical localization

Localization of the five transporters in the salamander retina is shown in Fig. 2. Like the rat retina [24,26] sEAAT1 is abundant, present in both the outer plexiform layer (OPL) and the inner plexiform layer (IPL), and labels primarily glial cells. The strongest staining is in the areas surrounding the photoreceptor cell bodies in the outer nuclear layer (ONL). This staining may represent glial cell processes which come close to photoreceptor synapses [27] but we cannot rule out the presence of sEAAT1 in photoreceptors as well. The cells stained in the inner nuclear layer (INL) largely consists of glial cell bodies, which are oblong in shape and course through much of the INL. Additional staining can be seen surrounding other cell bodies of the

Table 2
Summary of the pharmacology of salamander retinal glutamate transporter clones

Drug	SEAAT1		SEAAT2A		SEAAT2B		SEAAT5A	
	EC_{50} (μM)	I_{max}	EC_{50} (μM)	I_{max}	EC_{50} (μM)	I_{max}	EC_{50} (μM)	I_{max}
L-glutamate	25	(1)	40	(1)	110	(1)	43	(1)
D-glutamate*	≥10 000	0.45	≥10 000	0.13	≥10 000	0.11	≥10 000	0.32
L-aspartate	11	0.32	16	1.4	22	1.8	2.1	1.0
D-aspartate	16	0.15	22	1.4	42	1.0	5.2	0.92
THA	38	0.32	22	0.35	29	0.45	5.6	0.37
	K_i (μM)	I_{max}	K_i (μM)	I_{max}	K_i (μM)	I_{max}	K_i (μM)	I_{max}
Kainate	>1000	(0)	32	(0)	53	(0)	>1000	(0)
Dihydrokainate	>1000	(0)	13	(0)	19	(0)	>1000	(0)

EC_{50} and I_{max} are parameters derived from the Michealis-Menten equation, K_i is derived using the Schild equation. I_{max} is normalized relative to the response to L-glutamate.

INL but these are probably glial cell processes which have been shown to surround somata in the INL [24,28]. Somewhat sparse labeling of horizontal processes is present in the IPL and is likely to be the result of glial cell processes which are probably coming in close contact with glutamatergic synapses made there [24,28]. Staining is also intense in the endfeet of the glial cells but does not seem to be localized to ganglion cell membranes.

Localization of sEAAT2A is also intense around the photoreceptor cell bodies in the ONL, and may represent staining in glial cells or photoreceptors or both. In addition, other cell bodies in the inner nuclear layer stain for sEAAT2A. We believe that both amacrine and bipolar cells may possess sEAAT2A. This would explain the intense staining in the IPL, which unlike sEAAT1, shows nearly complete coverage. The staining is somewhat striated, being the most intense in the middle third of the IPL. Very little staining is seen in or around the ganglion cell bodies.

sEAAT2B is perhaps the most specific of the five transporters, being localized only in OFF (or hyperpolarizing) bipolar cells. Staining is most intense in the OPL. In addition, staining is present in the membranes surrounding cell bodies in the outer half of the inner nuclear layer. These appear to be bipolar cells as some staining of Landolt's clubs is seen above the OPL. Axons can be seen coursing through the INL and bright punctate staining is present in the outer half of the IPL.

Staining for sEAAT5A and sEAAT5B appear to be quite similar. Both are strongly present in the OPL, and label glial cells throughout the retina. Interestingly, unlike sEAAT1, the inside of the glial cells stain as well as the cell membrane. It is not clear whether this is an artifact due to the slicing procedure, although this staining pattern has been observed on every occasion. In addition to the glial staining, sEAAT5 is localized in most cell bodies in the retina. This is specific staining, as competing out the antibody eliminates all this staining. The staining of cell bodies in both the inner and outer nuclear layer is more clearly seen in sEAAT5B, although it is present in sEAAT5A as well. In addition, staining is present in and among ganglion cells.

3.3. Pharmacology and ionic dependence

Since the transport of glutamate is electrogenic, we can examine the functional properties of the five transporter clones using electrophysiological techniques. mRNA for each of the five transporters was injected individually into *Xenopus* oocytes, and two-electrode voltage clamp recordings were performed 3–7 days after injection. When held at negative potentials, the application of L-glutamate to an oocyte expressing any of the transporter clones except for sEAAT5B resulted in an inward current.

No current was ever observed in sEAAT5B injected oocytes, nor was there any significant uptake of ^{3}H L-glutamate above background, even though Western blots demonstrate that protein was present in these oocytes (data not shown). Several possible explanations may underlie the lack of a functional sEAAT5B: (i) sEAAT5B may not be functionally expressed in the oocyte expression system; (ii) an auxiliary protein such as a second subunit may be required; or (iii) sEAAT5B may itself be an auxiliary protein, and form heteromers with another transporter such as sEAAT5A. Because of the lack of functional expression, sEAAT5B has not been studied further.

The currents elicited by various concentrations of L-glutamate in a representative cell are shown in Fig. 3. As has been reported in other native and cloned transporters, the current is both voltage and dose-dependent, and inwardly rectifying, although an outward current at positive potentials was observed for all clones except sEAAT2B. As has been shown elsewhere [21,23], this outward current is the result of the passive influx of

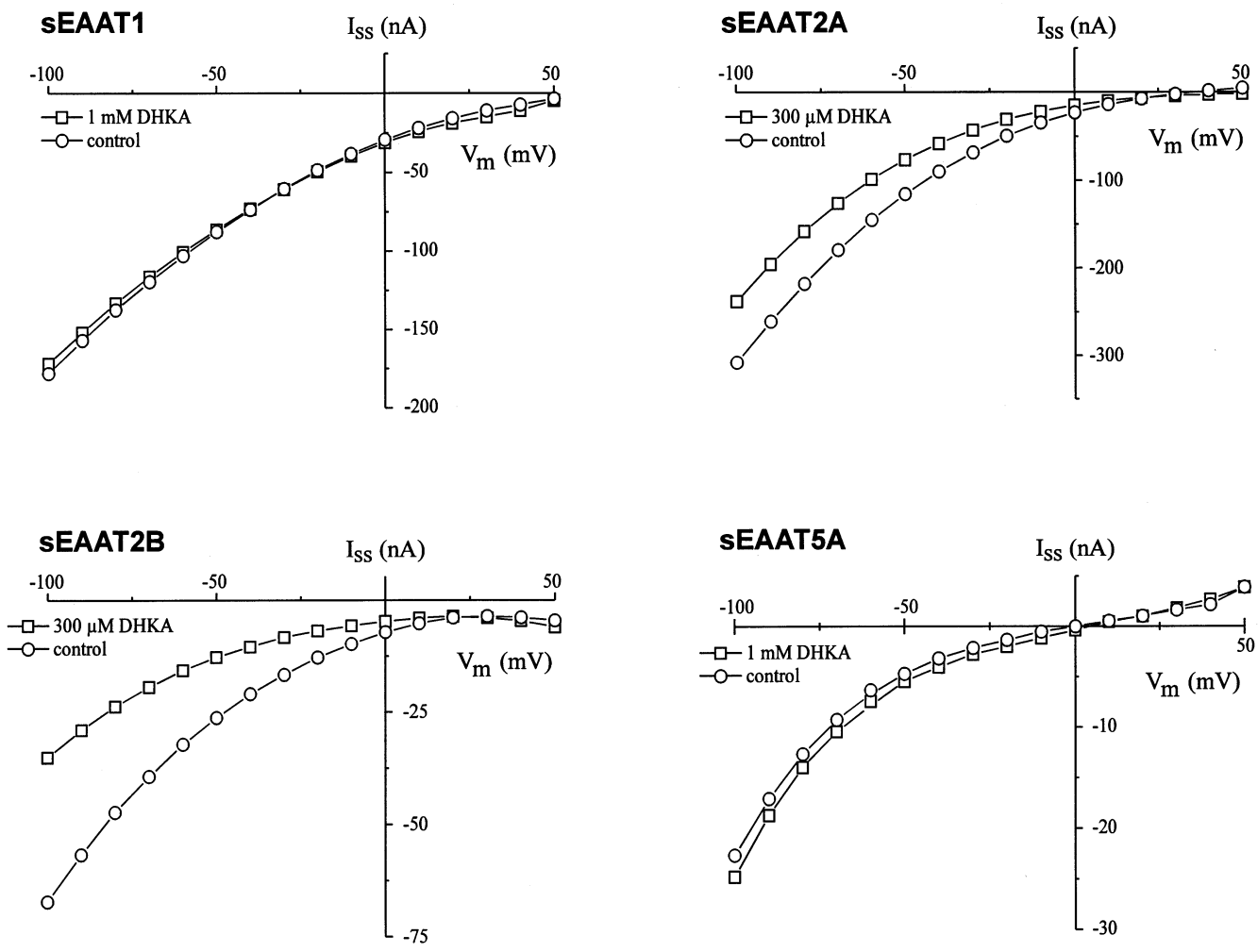

Fig. 5. Dihydrokainate reduces glutamate-elicited currents only in sEAAT2A and sEAAT2B. Plots of steady-state current (I_{ss}) elicited by either 100 μM L-glutamate alone (labeled 'control') or in the presence of 300 μM dihydrokainate (sEAAT2A and sEAAT2B) or 1 mM dihydrokainate (sEAAT1 and sEAAT5A) as a function of membrane potential (V_m). Each set of curves represents a typical cell. Similar results were obtained in four other cells for each subtype.

negatively charged chloride ions through the transporter-associated anionic conductance. The outward current is most prominent for sEAAT5A. Like the human homologue EAAT5 [15], both the outward and inward currents in sEAAT5A are largely the result of the anionic conductance.

From experiments of this kind, we can determine the affinity for various substrates by constructing dose-response curves at a given potential. Dose-response curves at -60 mV plotted from the average of five cells for each substrate are shown in Fig. 4. Smooth lines represent the Michaelis-Menten equation fit to the various data. The values for EC_{50} and I_{max} used for these fits are shown in Table 2. These experiments show that all four transporters respond stereospecifically for glutamate, but less so for aspartate. sEAAT2B and sEAAT5A have a significantly higher affinity for L-glutamate than either L- or D-aspartate, whereas sEAAT1 is a fairly poor transporter of aspartate. All four transporters transport the substrate inhibitor THA with a reasonably high affinity, but with an I_{max} that is considerably lower than for L-glutamate.

The non-substrate transporter blocker dihydrokainate (DHKA) partially inhibits the glutamate-elicited current to various degrees in retinal glial cells, bipolar cells, and both types of photoreceptors [6–8,29]. DHKA and the closely related molecule kainate (KA) specifically inhibit the EAAT2 subfamily of transporters in the micromolar range [13]. Consistent with this data, we found that 300 μM DHKA inhibits the response to 100 μM L-glutamate in sEAAT2A and sEAAT2B, whereas concentrations as high as 1 mM DHKA had no significant effect on sEAAT1 or sEAAT5A (Fig. 5). From Schild analysis studies, we have determined that the K_i for KA and DHKA are in the micromolar range for sEAAT2A and sEAAT2B, and in the millimolar range for sEAAT1 and sEAAT5A [25] (Table 2).

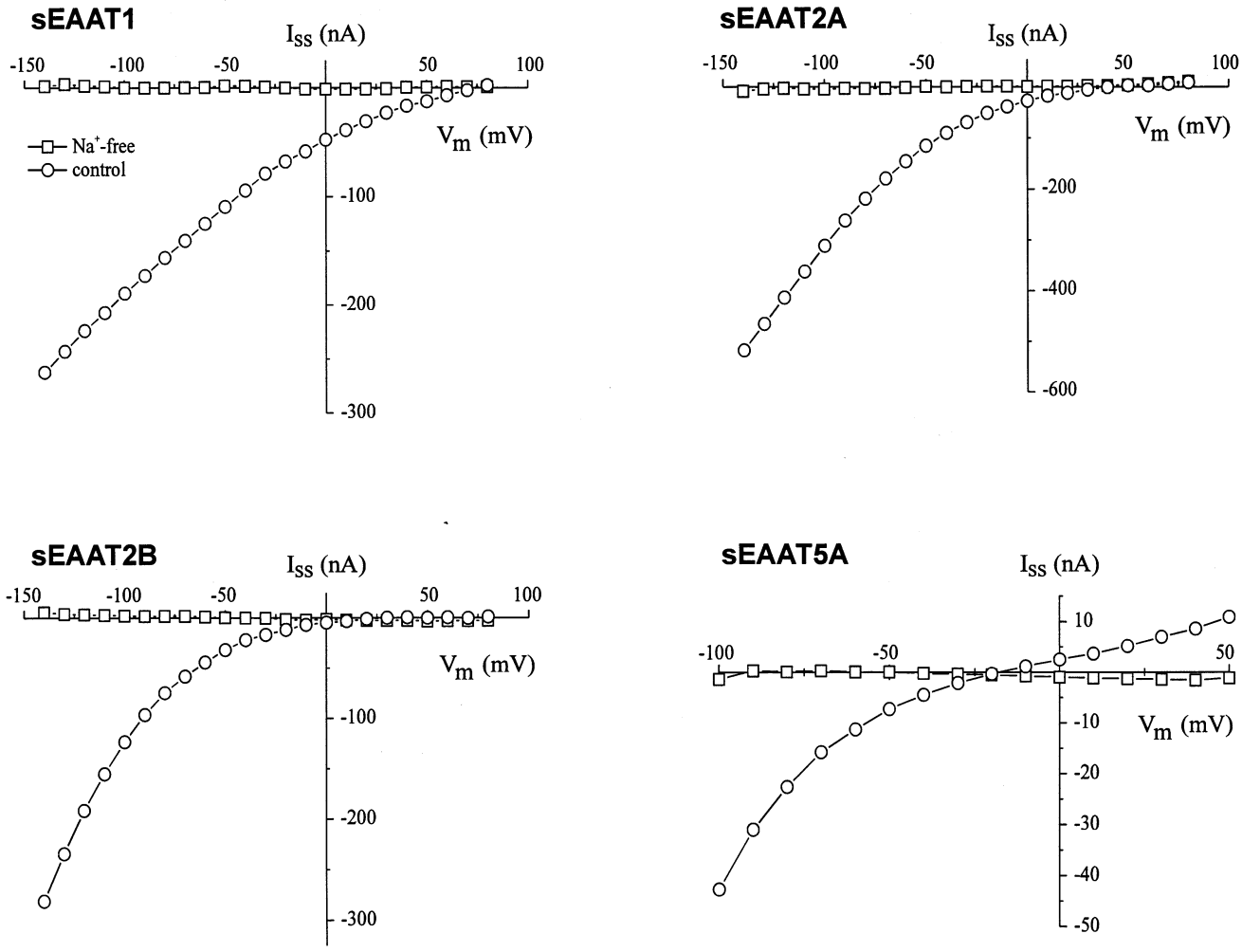

Fig. 6. Glutamate-elicited currents require the presence of extracellular sodium. Plots of steady-state current (I_{ss}) elicited by 100 μM L-glutamate in normal extracellular solution containing 96 mM NaCl (labeled 'normal') or in sodium-free extracellular solution containing 96 mM NMDGCl (labeled 'Na^+-free') as a function of membrane potential (V_m). Each set of curves represents a typical cell. Similar results were obtained in four other cells for each subtype.

All high affinity glutamate transporters in the native retinal cells as well as in the cloned transporters require the presence of extracellular sodium, since the movement of sodium down its electrochemical gradient supplies the energy that drives the transport of glutamate. Replacing sodium with N-methyl D-glucamine (NMDG) abolished the current elicited by 100 μM L-glutamate in all four salamander clones studied (Fig. 6).

The glutamate-elicited current in rod and cone photoreceptors and depolarizing bipolar cells has been shown to be largely carried by chloride ions [7,8,19]. In addition, retinal glial cells also show a small but measurable chloride current [21,22]. It has been demonstrated in cloned transporters that this current is the result of a thermodynamically uncoupled chloride conductance that is thought to be the result of chloride ions flowing through the transporter after glutamate and sodium have bound, but before they are released into the intracellular space [23]. This anionic conductance can be dramatically enhanced by replacing chloride with the more permeable anion nitrate [23]. As shown in Fig. 7, we found that external nitrate significantly enhanced the outward current for all four clones. Thus, all four cloned transporters possess an anionic conductance. The nitrate current declines at positive potentials for sEAAT2B. This may occur because the affinity for L-glutamate declines steeply with depolarization for sEAAT2B but not for the other three clones (unpublished observations).

4. Discussion

We have demonstrated that at least five different glutamate transporters are expressed in the salamander retina. A summary table indicating the localization and functional properties of the five transporters is shown

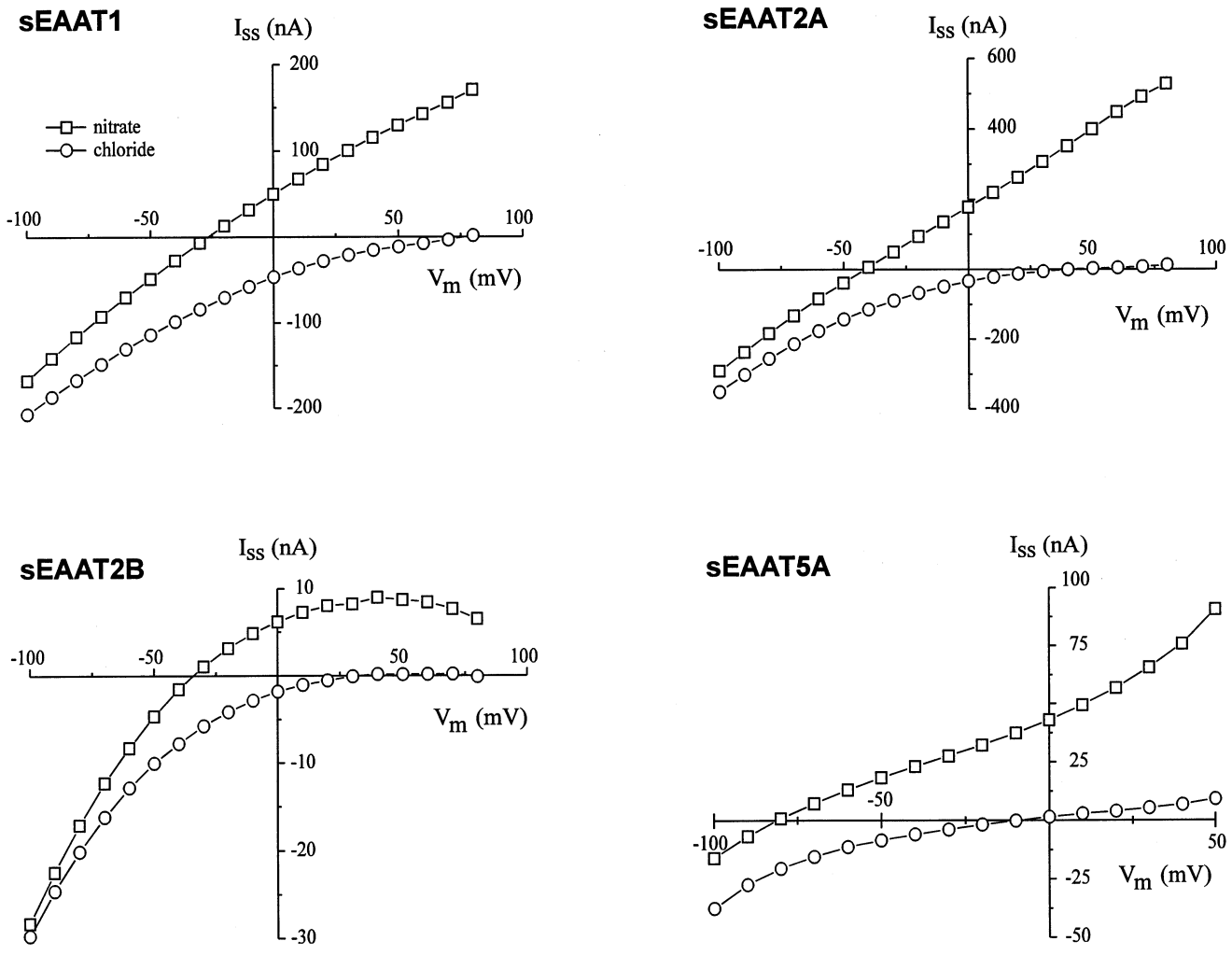

Fig. 7. Extracellular nitrate significantly increases the outward current elicited by glutamate at positive potentials. Plots of steady-state current (I_{ss}) elicited by 100 μM L-glutamate in normal extracellular solution containing 96 mM NaCl (labeled 'chloride') or in extracellular solution containing 96 mM $NaNO_3$ (labeled 'nitrate') as a function of membrane potential (V_m). Each set of curves represents a typical cell. Similar results were obtained in four other cells for each subtype.

in Table 3. As can be seen, transporters are found in almost every cell type in the retina: rods, cones, bipolar cells, amacrine cells, glial cells and ganglion cells. Although there is no evidence in salamander for glutamatergic amacrine cells, the presence of glutamate transporters in and around non-glutamatergic cells is not new. Both EAAT3 and EAAT4 have been shown to exist post-synaptically in GABAergic neurons in rat brain [30,31] and amacrine cells in the rat retina are reported to possess GLT-1 (rat EAAT2) [24].

The localization of sEAAT1 is strikingly similar to the localization of EAAT1 in rat retina [24,26]. In both studies, glial cells are predominantly stained. EAAT2 is localized in cone photoreceptors and in a subclass of amacrine and ON bipolar cells, but not in glial cells in the rat retina [24,32,33]. In contrast, sEAAT2A is found in glial cells, as well as amacrine, bipolar, and possibly photoreceptor cells in the salamander retina. It may be possible that an additional EAAT2 homologue exists in rat retinal glial cells. However, no one has yet probed the rat retina for additional glutamate transporters.

A second EAAT2 homologue, sEAAT2B, is localized only in OFF bipolar cells. This transporter appears to be both pre-synaptic and post-synaptic as dendrites, axons and axon terminals are all stained. A similar immunostaining of GLT-1 (rat EAAT2) in OFF bipolar cells was observed in macaque monkey retina [32,34]. The presence of a transporter in OFF bipolar cells may be due to the fact that these cells release glutamate tonically in the dark. Like photoreceptors, these cells release glutamate at tremendously high rates and thus have a greater need for clearing glutamate from the synaptic areas. Why a specific transporter would be required for such a function is unknown.

Table 3
Summary of the properties of the glutamate transporter subtypes from salamander retina

Salamander clone	Chloride conductance	KA sensitivity	Relative abundance	Salamander retina localization
SEAAT1	Some	mM range	High	Glial, photoreceptors?
SEAAT2A	Some	μM range	High	Glial, photoreceptors, bipolar cells, amacrine cells
SEAAT2B	Some	μM range	Medium	OFF bipolar cells
SEAAT5A	>90%	mM range	Medium	Glial, photoreceptors, bipolar cells, amacrine cells
SEAAT5B	?	?	Low	Glial, photoreceptors, bipolar cells, amacrine cells, ganglion cells

Abundance values were estimates based upon data obtained with western and northern blots.

In addition to EAAT1 and EAAT2 homologues, we found two different homologues of the transporter EAAT5, which has been shown to be present in the human retina [15]. The current through sEAAT5A is carried largely by chloride ions, and so this transporter most closely resembles the transporter described in rods, cones and bipolar cells. However, the transporter is not uniquely localized to these cell types but appears to be present in glial, amacrine and ganglion cells as well. If this is the transporter responsible for the behavior present in photoreceptors and bipolar cells, then it may be up-regulated in these cells, or the presence of other more traditional glutamate transporters in glial, amacrine and ganglion cells dominates the response in these cells. It is also possible that a closer examination of the glutamate response in amacrine and ganglion cells may reveal a small but measurable sodium-dependent chloride conductance due to the presence of sEAAT5A.

We were unable to measure any glutamate uptake or glutamate elicited currents in sEAAT5B. Additionally, the pattern of localization of sEAAT5B looks strikingly similar to the staining pattern of sEAAT5A. A trivial explanation would be that *Xenopus* oocytes are incapable of expressing functional sEAAT5B. However, we were able to detect the presence of sEAAT5B in crude membrane preparations from injected cells using immunoblots. One possibility is that sEAAT5B is not a functional transporter on its own, but forms heteromers with sEAAT5A. Perhaps even a differential stoichiometry of coupling between sEAAT5A and sEAAT5B may explain the predominant presence of a chloride conductance in some cell types but not others.

Transporter co-localization has been shown in rat brain [18,35] and also occurs in the salamander retina. For example, we found that glial cells contain all of the five transporters except sEAAT2B. This result explains the partial block of glutamate uptake by micromolar concentrations of dihydrokainate [21,29]. Photoreceptors also must contain more than one transporter, since the glutamate transport current is largely carried by chloride ions, and is partially blocked by dihydrokainate [6,20]. In fact, this may be evidence for the existence of heteromers between sEAAT5 and sEAAT2A since sEAAT5A, the chloride channel-like transporter, is not dihydrokainate sensitive whereas sEAAT2A is sensitive to dihydrokainate.

Why are so many transporters needed in the salamander retina, or in any neural tissue? First, the various transporters do not function alike. Such functional differences may be important for the specific roles that each transporter plays. For example, the glutamate-elicited current in sEAAT5A is carried largely by chloride ions, in contrast with sEAAT1 and the two EAAT2 homologues. Thus, sEAAT5A (and possibly sEAAT5B) may function more as post-synaptic receptors than as removers of glutamate from the synaptic areas. sEAAT2B has a relatively low apparent affinity for glutamate, and therefore may allow for higher synaptic concentrations of glutamate in the synaptic areas in which it is found. Second, it is clear that at least some cells possess multiple transporter subtypes. Whether or not these transporters form heteromers, having multiple transporters may allow for a kind of 'fine tuning' of the way in which a cell will respond to extracellular glutamate. Finally, the possibility exists that the various transporter subtypes within a particular cell may be localized differentially. Transporters may appear exclusively in pre-synaptic or post-synaptic regions, for example. In this way, each transporter is localized to the region where its particular function is most suitable.

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